



## Optimization of lactic fermentation for extraction of chitin from freshwater shrimp waste

Andressa Caroline Neves<sup>1</sup>, Cristina Zanette<sup>2</sup>, Schaiana Tamara Grade<sup>1</sup>, Jéssica Vanessa Schaffer<sup>1</sup>, Helton José Alves<sup>1</sup> and Mabel Karina Arantes<sup>1\*</sup>

<sup>1</sup>Laboratório de Catálise e Produção de Biocombustíveis, Universidade Federal do Paraná, Rua Pioneiro, 2153, 85950-000, Palotina, Paraná, Brazil. <sup>2</sup>Universidade Estadual do Centro Oeste, Guarapuava, Paraná, Brazil. \*Author for correspondence. E-mail: mabelarantes@gmail.com

**ABSTRACT.** Freshwater shrimp shells from the shrimp farming activity in tanks, were processed for biological extraction of chitin, by fermentation with *Lactobacillus plantarum* isolated from meat products, offering an advantageous demineralization and deproteination of the residue, replacing the chemically. Deproteinization was obtained approximately 99% and demineralization of up to 87% using batch fermentations with a maximum of 72 hours and the use of simple strategies such as pH adjustment and reinoculation. The performance of chitin was about 40% greater than in the chemical extraction and the results indicate an interesting method in the process of production of chitosan, where the biopolymer chitin is precursor.

**Keywords:** shrimp shells, chitosan, *Lactobacillus plantarum*, bioprocess.

## Otimização de fermentação láctica para extração de quitina de resíduos de camarão de água doce

**RESUMO.** Carapaças de camarão de água doce provenientes da atividade de carcinicultura em tanques foram processadas para extração biológica de quitina, por meio da fermentação com *Lactobacillus plantarum* isolado de produtos cárneos, oferecendo uma via vantajosa de desmineralização e de desproteínização do resíduo, em substituição à via química. Obteve-se desproteínização de cerca de 99% e desmineralização de até 87% por intermédio de fermentações em batelada com duração máxima de 72 horas e o uso de estratégias simples, como ajuste de pH e reinoculação. O rendimento de quitina foi cerca de 40% maior do que na extração química, e o conjunto dos resultados indica um método interessante no processo de produção de quitosana, em que o biopolímero quitina é precursor.

**Palavras-chave:** carapaças de camarão, quitosana, *Lactobacillus plantarum*, bioprocessos.

### Introduction

The use of wastes as economically feasible raw materials can reduce their environmental impact and add sustainability to industrial and agro-industrial activities. The residue approached in this paper clearly exemplifies this scenario: shrimp shells, fishing industry rejects, are potentially polluting due to the decomposition of their organic material when disposed of in an inappropriate manner in dumps, but are also an important source of the raw material, chitin (2-Acetamide-2-Deoxy-D-glucose), precursor of the biopolymer chitosan (2-amino-2-Deoxy-D-glucose and 2-Acetamide-2-Deoxy-2-D-glucose). Chitosan provides a high value due to its broad spectrum of applications from the food industry and cosmetics to biotechnological and biomedical areas. It has been used, for example, in drug delivery and gene delivery systems (Harish & Tharanathan, 2007;

Jayakumar et al., 2010) and in cases where hypolipidemic, hypocholesterolemic and bactericidal effects, are desired (Vishu, Varadaraj, Lalitha, & Tharanathan, 2004). Recently the use of chitin and chitosan for producing carbon quantum dots (CQDs) has also been reported, in an emerging new family of nanocarbon materials, functionalized for applications in solar cells (Briscoe, Marinovic, Servilla, Dunn, & Titirici, 2015).

Shrimp shells are composed of the biopolymer chitin aimed proteins, minerals and carotenoid pigments, constituents that once extracted from the residue can be availed. Chitin is 15-20% of the mass of the residue and can be converted to chitosan through deacetylation, which is the conversion of acetamide groups (-NHCOCH<sub>3</sub>) to amino (-NH<sub>2</sub>) groups (Tolaimate, Desbrieresb, Rhazia, & Alaguic, 2003). The development of optimized techniques

from the preparation of shells, through chitin extraction to chitosan production aims to obtain chitosan with specific characteristics depending on the type of application, with emphasis on the degree of deacetylation and the molar mass of the biopolymer (Yue, Yao, & Wei, 2009; Arantes et al., 2015).

World production of shrimps, (marine and freshwater) was estimated at approximately 4 million tons in 2013, and freshwater shrimp farming contributes strongly to these numbers, having increased about twelve-fold in the last decades (Food and Agriculture Organization of the United Nations [FAO], 2015). In Brazil, although the production of freshwater prawns has been variable, factors such as increasing demand, improved prices and production chain organization (Marques & Moraes-Valenti, 2012) have ensured the creation and consequently the availability of residues (shells) rich in chitin, and other components. The main commercially produced species are *Macrobrachium rosenbergii* and native species as *M. amazonicum*, *M. acanthurus* and *M. carcinus* have been cultivated in the northeastern region of Brazil (Valenti, 2007).

The chemical process most commonly employed for the extraction of chitin from shrimp waste uses acidic solutions and strong bases for demineralization and deproteinization of the residue, respectively. Chitin is extracted with high efficiency, however there are disadvantages such as the generation of new waste (acidic and basic solutions containing proteins and minerals) and the non-exploitation of possible by-products (carotenoid pigments and protein and mineral content).

In this context the biological extraction of chitin presents an important opportunity for the utilization of shrimp waste, using the fermentation of shrimp shells with micro-organisms such as *Lactobacillus acidophilus*. Demineralization and deproteinization are consequences of the production of organic acids (e.g. lactic acid) and of the action of proteases of the microorganism, respectively. In the literature several papers have described the fermentation of shrimp farming residues from salt water: Use of *L. helveticus* was shown by Arbia, Adour, Amrane and Lounici (2013); Duan et al. (2012) used *L. acidophilus* isolated from the residue itself; Rao and Stevens (2005; 2006) used *L. plantarum*. In addition, other bacteria or biological methods can also be found in the literature involving the use of different strains of *Bacillus* (Ghorbel-Bellaaj, Younes, Maâlej, Hajji, &

Nasri., 2012; Ghorbel-Bellaaj et al., 2013) and *P. aeruginosa* (Ghorbel-Bellaaj et al., 2011).

A combination of chemical and biological methods can also be used: Manni, Ghorbel-Bellaaj, Jellouli, Younes and Nasri (2010) achieved demineralization by acid treatment (HCl 1,5 M, 6 hours) and the deproteinization by using extracellular proteases isolated from *B. cereus*. A strategy of successive fermentations with different strains was adopted by Zhang and collaborators (Zhang, Jin, Deng, Wang, & Zhao, 2012; Zhang et al., 2014), comprising two fermentation steps with *S. marcescens* and *L. plantarum*.

It is interesting to note, reviewing published studies, that different species of shrimp among the aforementioned saltwater species (*Parapenaeus longirostris*, *Metapenaeus monôceros*, *Penaeus vannamei*) exhibit different contents of ash (18 to 46%), proteins (25 to 41%) and chitin (25 to 27%), which along with the acidification and protease production ability the bacteria used can lead to different results in terms of demineralization, deproteinization and final yield. In the case of freshwater shrimp information on its composition or on the extraction of chitin is not currently available in the literature. As freshwater shrimp farming activity has expanded, it has become more important to characterize the waste and develop processes to exploit it as a resource, to prevent environmental problems and obtain high added value products such as chitosan. Following the earlier work of our group on the chemical extraction of chitin and chitosan production (Arantes et al., 2015) the present work proposes the use of a fermentation method for extracting chitin capable of replacing the traditionally used chemical method.

In this study residues of freshwater shrimp shells of the species-*Macrobrachium rosenbergii* from the region West of Parana (PR) State were used as a source of chitin for fermentation with *Lactobacillus plantarum* isolated from sausages. Chitin extracted by this process was subjected to chemical deacetylation. Chitosan production and the final product were compared for the two types of chitin extraction, chemical and biological.

## Material and methods

### *L. plantarum* isolation and characterization of suspended cultivation

The strain used in this study was isolated from samples of sausages traditionally produced by

spontaneous fermentation. The isolate was purified in Agar of Man, Rogosa and Sharpe (MRS) (DIFCO, Code 288210) and preserved at  $-18^{\circ}\text{C}$  in MRS broth (DIFCO, 288130) with 20% ( $\text{V V}^{-1}$ ) sterile glycerol (Samelis, Maurogenakis, & Metaxopoulos, 1994). The isolate was identified by the system API 50 CHL (BioMerieux, Marcy L'Etoile, France) and a Multiplex PCR technique with specific primers as for *Lactobacillus plantarum*. The isolate was biochemically characterized as homofermentative and selected for study on the basis of its ability to bring about rapid acidification.

Suspension cultures were performed to evaluate the profile of growth variation of pH and lactic acid production in MRS medium. Pre-inoculum prepared by overnight incubation at  $35^{\circ}\text{C}$  were inoculated in MRS at a proportion of 4% ( $\text{V V}^{-1}$ ) in agitated cultures at  $35^{\circ}\text{C}$  for about 30 hours. Aliquots were removed at each time point to evaluate cell growth by measuring optical density at 520 nm, measurement the pH of the culture and determine the production of lactic acid by titration with standard NaOH.

#### Carapace processing and extraction of chitin

*Preparation of shrimp shells:* The shrimp shells were collected on harvest of shrimps in producing units in the region West of PR and frozen at  $-20^{\circ}\text{C}$  until cleanup, which consisted of washing in running water and drying at  $60^{\circ}\text{C}$  for 12 hours. After drying the shells were ground in a mixer for 10 minutes and milled in a ball mill for 5 hours.

*Extraction of chitin by fermentation:* Strains of *L. plantarum* were inoculated in MRS medium at pH 6.2 (a volume of 20 mL) and incubated overnight at  $35^{\circ}\text{C}$ . The next morning this inoculum was added to the tests, conducted as follows: Control Test (C): Ground shrimp shells ( $33\% \text{ w V}^{-1}$ ), sucrose solution 20% ( $50\% \text{ V V}^{-1}$ ) and inoculum ( $50\% \text{ V V}^{-1}$ ), of a culture with cell concentration of about  $10^6 \text{ CFU mL}^{-1}$ ; Test with reinoculation ( $\text{C}_r$ ): Test conditions the same as the control, but reinoculated every 24 hours (20 mL of inoculum); Test with reinoculation and pH adjustment ( $\text{C}_{r,\text{pH}}$ ): maintained the initial conditions with pH adjustment to values between 5–6 with small volumes (less than 0.5 mL) of glacial acetic acid, at the initial time point and at time intervals of up to 12 hours. All trials were performed with orbital agitation (125 rpm) at  $35^{\circ}\text{C}$ . At the end of each test the solid fraction was washed and depigmented with  $\text{NaClO}$  1 ( $\text{w V}^{-1}$ ), once at  $40^{\circ}\text{C}$ , followed by washing to neutral pH (wash water) and dried at  $60^{\circ}\text{C}$  for 24 hours.

*Chemical extraction of chitin:* for comparison purposes between the chemical and fermentation processes for extracting chitin, chemical demineralization through treatment with 0.55 M HCl was performed, at the proportion of 20% ( $\text{w V}^{-1}$ ) chitin: HCl, with three washes of 20 minutes each, at room temperature. After the third washing, the material was washed to neutral pH with vacuum filtration and deproteinated with three washes of 20 minutes at  $80^{\circ}\text{C}$  with 0.3 M NaOH. After washing to neutral pH the material was dried at  $60^{\circ}\text{C}$  for 24 hours.

#### Production of chitosan

To obtain chitosan by deacetylation of chitin, 6.7% ( $\text{w V}^{-1}$ ) of chitin in 15 M NaOH solution was agitated in vials in a rotating incubator for 168 hours at room temperature. Then the material was washed to neutral pH (wash water), filtered and dried at  $60^{\circ}\text{C}$  for 24 hours for further characterization.

#### Physico-chemical characterization of fermentation and of chitin and chitosan

Samples collected during each test were analyzed for production of lactic acid by titration with 0.1 M NaOH (default second technique Association of Official Analytical Chemists [AOAC], 2012), discounting the volume of base required to neutralize the organic acid added, pH value (pH meter) and the density of microorganisms (Pour Plate).

The efficiency of demineralization in the isolation of chitin was evaluated by ash content, determined by burning the samples in a crucible at  $600^{\circ}\text{C}$  in an electric muffle furnace. For the evaluation of deproteination the protein content was measured by using an adaptation of the standard Biuret protein assay: samples before and after fermentation were exposed to alkaline deproteination and the protein content in the supernatant was measured by the Biuret assay, the method adapted from Rao and Stevens (2006). To check the reliability of this method selected results were compared with those obtained by the Kjeldahl method (Rao & Stevens, 2005). The calculations of demineralization (% DP) and deproteination (% DM) were conducted in accordance with Rao and Stevens (2006), taking into account the mass of protein and ash before and after each fermentation (Rao & Stevens, 2006).

The average degree of acetylation (GA) and deacetylation (GD) of chitosan samples was obtained by conductimetric titration, as described by Santos et al. (2009). To check the reliability of the method

selected results were compared with the GA determined using a Bruker Avance III of 9.4 Tesla (400 MHz for the frequency of hydrogen).

For determination of the characteristic groups of chitin and chitosan Infrared Spectroscopy (FTIR) as described by Arantes et al. (2015) was performed. The crystalline/semi-crystalline structures of the material were analyzed by X-Ray diffraction (XRD) using a Siemens Kristalloflex diffractometer (Cu K  $\alpha$  radiation with  $\lambda = 1.54 \text{ \AA}$ , 40 kV voltage and 40 mA current), with continuous scanning in the interval of  $5^\circ < 2\theta < 50^\circ$  and a scan speed of  $1 \text{ min}^{-1}$ .

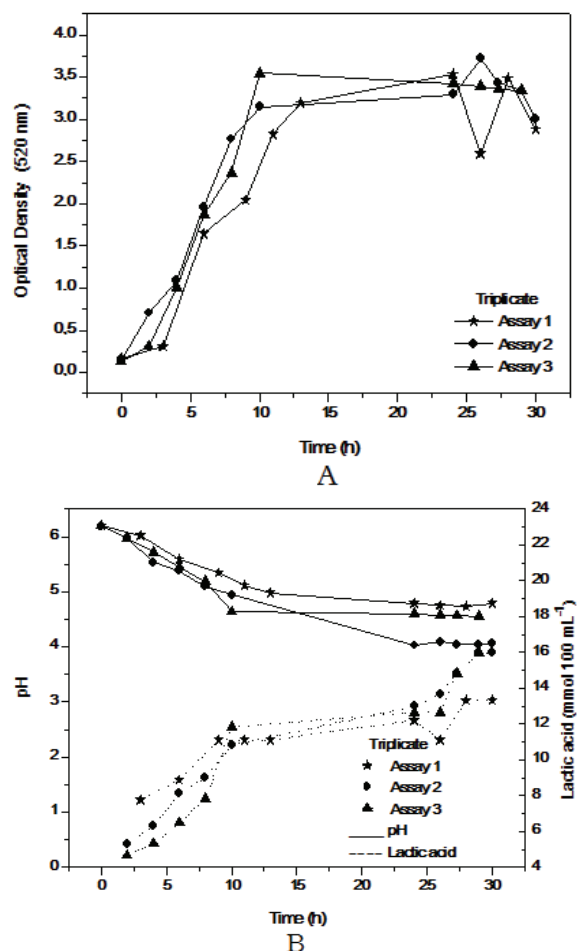
## Results

### Characterization of *L. plantarum* culture and fermentation of waste shrimp

The crops of *L. plantarum* in suspension showed typical characteristics of acidophilus bacteria, with excellent performance: from a bacterial population close to  $0.15 \text{ OD mL}^{-1}$  the maximum concentration was around  $3.6 \text{ OD mL}^{-1}$  in approximately 10–11h, the time required for the pH minimum to be reached (pH 4.0). With respect to the production of lactic acid, an increase was observed throughout the entire culture, until its completion. The maximum lactic acid was produced between 10–11 h, in parallel with the maximum biomass production (Figure 1).

Fermentation of shrimp shells with *L. plantarum* conducted initially without any intervention during cultivation led to unsatisfactory results for DM and PD (Table 1). The efficiency of fermentation depends on factors such as the quantity of inoculum, initial pH and pH during fermentation. The pH of the MRS medium was 6.2 and was unfavourable to acidophilus bacterial metabolism. The inoculum size and viability of microorganisms during fermentation affects the deproteinization as a result of the action of extracellular proteases.

The reinoculation of microorganisms every 24 hours during the culture led to an improvement in deproteinization (83 versus 99%), indicating the greater availability of extracellular proteases due to higher bacterial populations. However, the increase in demineralization was small, which motivated the adoption of pH adjustment at various stages of cultivation, a strategy that led to a demineralization of up to 87% (average). This increase should not be attributed only to the addition of small amounts of acetic acid (less than 0.5%), but to favor the bacterial metabolism in order to produce lactic acid, which is demonstrated by the concentration of the acid for test adjustment pH (Figure 2).

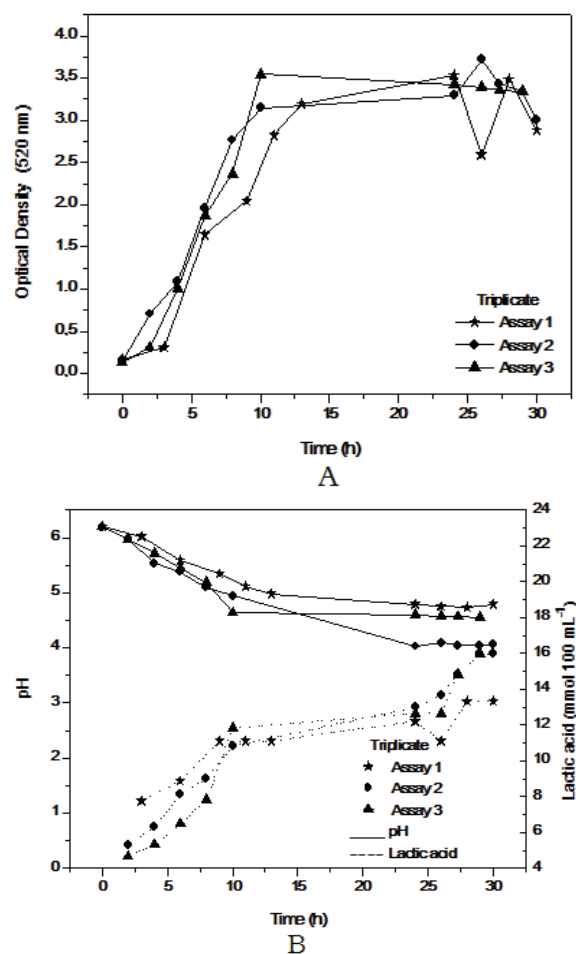


**Figure 1.** Growth curve (A), pH and lactic acid (B) for crops of *L. plantarum* in MRS (triplicate).

### Shrimp waste characterization

The species *Macrobrachium rosenbergii* is one of the most used species for freshwater shrimp cultivation. In the Western region of PR shrimp production in tanks in consortium with fish has been growing, offering shrimp shells among the waste, which have been identified and processed in present lab. The average levels of minerals and proteins obtained from clean and dry shrimp shells were 41 and 24%, respectively, while the average moisture content was 25%. The chitin content, calculated from the total extraction of minerals and ash with acids and bases was about 35% and results obtained in our laboratory for chemical extraction of chitin ranged between 20% (Arantes et al., 2015) and 35% (current data), varying according to the filtering techniques, which can lead to loss of solid material in the washes. Works with freshwater shrimp or *M. rosenbergii* for comparison purposes were not found but for the marine species cited in this paper these results are comprised between the displayed values: can

cite for example the marine species *Parapenaeus longirostris*, *Metapenaeus monóceros*, *Penaeus vannamei*, and other species of the genus *Penaeus* for which values are given enough variables, especially protein (25 to 41%) and mineral content (ash content 18 to 46%) (Arbia et al., 2013; Duan et al., 2012; Ghorbel-Bellaaj et al., 2012; Ghorbel-Bellaaj et al., 2013; Ghorbel-Bellaaj et al., 2011). From the results obtained for ash and protein content before and after fermentation, demineralization (DM) and deproteinization (DP) of carapace through fermentation with *L. plantarum* under different strategies were determined (Table 1).

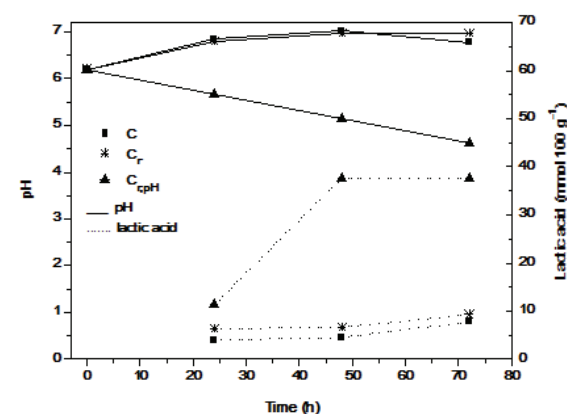


**Figure 1.** Growth curve (A), pH and lactic acid (B) for crops of *L. plantarum* in MRS (triplicate).

### Chitin characterization

The infrared spectra obtained for the fermentation product, chitin and chitosan (Figure 3) reveal bands characteristic of the biopolymers and the differences between them: an axial stretch band of OH between 3440 and 3480 cm<sup>-1</sup>, which appears superimposed on N-H stretching band, for chitin and chitosan; a CO axial deformation of amide I

(between 1650 to 1654 cm<sup>-1</sup>), more intense for chitin, as well as N-H of amide II (1558 cm<sup>-1</sup>); an axial deformation of -CN amide (circa 1420 cm<sup>-1</sup>), more intense in chitin while axial deformation and -CN of amino groups (circa 1380 cm<sup>-1</sup>) is more intense in chitosan. In addition, there were bands of polysaccharide structures (in the region of 1128 – 1157 cm<sup>-1</sup>) and symmetrical angular deformation of CH<sub>3</sub> (between 1323 to 1383 cm<sup>-1</sup>).



**Figure 2.** Variation of pH and production of lactic acid in the fermentation of shrimp shells with *L. plantarum* under different conditions (C: without intervention; C<sub>r</sub>: with reinoculation; C<sub>cpH</sub>: with reinoculation and pH adjustment).

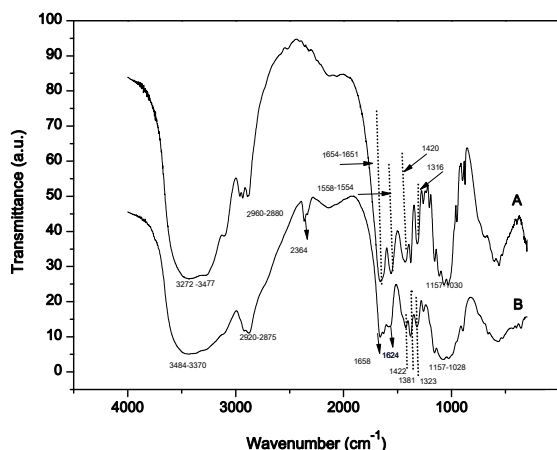
**Table 1.** Comparisons between features of the trials and results of demineralization and deproteinization of the residue.

	% DP	% DM	Lower pH reached	Maximum Production lactic acid (mmol. 100 mL <sup>-1</sup> ; mmol. 100g <sup>-1</sup> )
Cultivation without shrimp waste	-	-	4.0	16.0
Shrimp waste fermentation: Control (C)	83	57	6.8	*7.8
Shrimp Waste fermentation: Control + reinoculation (C <sub>r</sub> )	99	59	7.0	9.3
Shrimp waste fermentation: Control + reinoculation + pH adjustment (C <sub>cpH</sub> )	99	87	4.6	37.7

DM: demineralization; DP: deproteinization

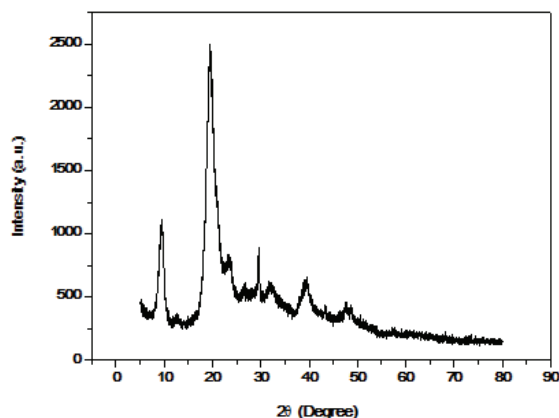
Evaluation of the fermentation product by DRX (Figure 4) indicates amorphous halos of  $\alpha$ -chitin in 2 $\theta$  = 9 and 19° which are typically observed in this biopolymer, and the presence of minerals characterized by crystalline peaks at 2 $\theta$  = 23.2, 29.5 and 39.6°, typical of the crystalline phase of calcium carbonate (CaCO<sub>3</sub>) (Silva & Debacher, 2010; Rezaei, Mohadesi, & Moradi, 2013). Between 30.5 and 32.5° small signs characteristic of amorphous calcium phosphate (Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>) are observed. It is worth noting that both calcium carbonate and phosphate minerals are commonly found in the shells of shrimp. Generally, chitins obtained by

chemical methods have only efficient amorphous halos of  $\alpha$ -chitin, with a main characteristic peak at  $19^\circ$  (Andrade, Ladchumananandasivam, Rocha, Belarmino, & Galvão, 2012).



**Figure 3.** FTIR spectra of chitin extracted by fermentation (A) and chitosan obtained from chitin extracted chemically (B).

The diffractogram in Figure 4 is consistent with the type of chitin obtained by fermentation, since demineralization was incomplete and peaks attributed to minerals present initially in the carapace of shrimps still remain.



**Figure 4.** Diffractogram of chitin extracted by fermentation.

#### Production of chitosan from biological and chemically extracted chitins

Samples of chemically extracted chitins or those obtained by fermentation were submitted to deacetylation as described earlier, to obtain chitosan with GD exceeding 75%. Chemically extracted chitin showed % DP and % DM of about 100% and the fermentation sample showed DP of 99% and DM of 87%. After the deacetylation reaction and recovery of neutral and dry sample, it was found through testing of solubility in organic weak acid

solution that, in the case of chitin extracted by fermentation, the deacetylation reaction did not occur efficiently, which is why the material was insoluble, or had very low solubility, which was not quantified. It is seen that solubility is due to protonation of the amino group ( $-\text{NH}_2$ ) in an acid medium when these are present in large quantities in relation to the acetamide groups ( $-\text{NHCOCH}_3$ ), naturally occurring in chitin. Once insoluble, the degree of acetylation may not be accurately determined by conductimetric titration.

However, the present study evaluated only one method of deacetylation common in our lab due to its simplicity of use at room temperature, with easy assembly, etc, and its reproductive efficiency in deacetylation of chemically extracted chitin. Deacetylation reactions with use of heat, acid reflux, producing more intense conditions should be evaluated for efficient deacetylation of material, even in the presence of mineral content. Rao and Stevens (2005) for example used deacetylation reactions of 48h at  $50^\circ\text{C}$  with NaOH solution 50 (w  $\text{V}^{-1}$ ), where for samples of chitin with a maximum of 88% DM, GD values were obtained between 81 and 84%.

#### Discussion

Traditional fermentations depend on naturally occurring microorganisms in the substrate or materials involved in the process such as tools, equipment or human handling. Inoculation of suitable lactic acid bacteria ensures rapid acidification and eventual predominance of desired micro- flora able to conduct fermentation processes (Rao, Munoz, & Stevens, 2000). As noted by Rao et al. (2000) fermentation of shrimp waste with *L. plantarum* inoculum resulted in a high-quality protein liquor output when compared with solely acid fermentation (Shirai et al., 2000).

The extraction of chitin from freshwater shrimp waste by fermentation may present as a limitation the occurrence of smaller % DM and % DP values when compared to the chemical treatment that has about 100% efficiency (Arantes et al., 2015). This fact is also noted in the literature for shrimp waste from marine species where 98% DM and 78% DP after 10 days of fermentation with *L. helveticus* (Arbia et al., 2013); 91% DM and 97% DP, after 7 days of fermentation with *L. acidophilus* (Duan et al., 2012); DM ranging from 63 to 81% and DP of 60 the 83%, with *L. plantarum* (Rao & Stevens, 2005; 2006). After testing six strains of *Bacillus*, (Ghorbel-Bellaaj et al., 2012; Ghorbel-Bellaaj et al., 2013) found the best

results were obtained with *B. cereus* and *B. pumilis*, with DM up to 88% and DP up to 95%. Using *P. aeruginosa*, after 5 days of cultivation 96% DM and 89% DP was obtained (Ghorbel-Bellaaj et al., 2011). Using the strategy of successive fermentations with different strains, *S. marcescens* and *L. plantarum*, (Zhang et al., 2012; Zhang et al., 2014) obtained in a total time of 6 days 93% of DP and 95% of DM.

Many factors, such as inoculum size, initial pH value, carbon concentration and carbon/nitrogen ratio have been reported to influence the fermentation process and consequently demineralization efficiency (Choorit, Patthanamane, & Manurakchinakorn, 2008). However, the adopted strategy led to the almost complete deproteinization (99%), which surpassed the values obtained by the researchers mentioned above. However a limitation of the method remains, in common with these works, because of the incomplete demineralization of the material.

Even if it is necessary to develop a step to complete the demineralization of the material, by optimizing the fermentation (duration, interventions, etc), either through a chemical treatment, more soft or using a smaller scale than for chemical demineralization, the biological method proposed is beneficial for the following reasons: reduced generation of residues, where processing of 20 g of shell by chemical method consumes 1.2 L of 0.55 M HCl and an equal volume of 0.33 M NaOH, containing extracted proteins and minerals, plus about 10 to 14 L of distilled water used for washing until neutral pH; the possibility of utilization of waste liquid (liquor) in brewing (about 90% of the initial volume of the test) since it contains hydrolyzed protein, unused sugars, minerals, lipids, components of the MRS, *Lactobacillus* in suspension and fermentation metabolites. Rao and Stevens (2005) characterized the liquor resulting from the fermentation of marine shrimp waste and observed a high content of amino acids in amounts significantly exceeding those found in food such as egg and milk powder. The use of the CSF as an additive for feed, biofertilizers or other applications deserves evaluation.

In favor of the biological method, the reduction of losses of chitin in the demineralization and deproteinization steps should also be taken into account. This is due to many washings and filtrations for removal of acid and base causing loss of chitin, which can reduce the chitin yield from 35 to 20%, as seen in the results obtained in the laboratory.

In addition, the use of fermentation for extracting chitin may allow recovery of carotenoid pigments from the cerebrospinal fluid, which adds economic value to the process. Among the pigments found in shrimp waste astaxanthin is the most abundant carotenoid (Ogawa et al., 2007) and the fact the fermentation does not use or minimizes the use of acid, base or conditions that may degrade carotenoids makes the process even more interesting. Because of the high content of the ketocarotenoid astaxanthin (3,3'-dihydroxy-bb-carotene-4-4'-dione), one of the most powerful natural antioxidants, the waste could serve a multipurpose role, being a good protein source for human nutrition, and contributing nutraceutical compounds.

## Conclusion

This work shows a microbiological method for the extraction of chitin from freshwater shrimp shells, capable of replacing the traditional chemical method. High percentages of deproteinization (99%) and demineralization (87%) of shrimp shells were obtained by fermentation with *Lactobacillus plantarum* for three days. However, the reaction conditions should be optimized in order to obtain a chitin free of mineral, thus avoiding future problems during the process of deacetylation. This 'green' route reduces the consumption of chemical reagents and allows a greater recovery of chitin, constituting an interesting alternative process for the production of chitosan.

## Acknowledgements

The authors are grateful to Professor Eduardo L. C. Ballester, Aquaculture Research Group and sustainable development, the UFPR.

## References

- Andrade, S. M. B., Ladchumananandasivam, R., Rocha, B. G., Belarmino, D. D., & Galvão, A. O. (2012). The use of exoskeletons of shrimp (*Litopenaeus vannamei*) and crab (*Ucides cordatus*) for the extraction of chitosan and production of nanomembrane. *Materials Sciences and Applications*, 3(7), 495-508.
- Association of Official Analytical Chemists [AOAC]. (2012). *Official methods of analysis of AOAC International* (19th ed.). Arlington, VA: Association of Analytical Communities.
- Arantes, M. K., Kugelmeier, C. L., Cardozo-Filho, L., Monteiro, M. R., Oliveira, C. R., & Alves, H. J. (2015). Influence of the drying route on the depolymerization and properties of chitosan. *Polymer*



- Engineering Science*, 55(9), 1969-1976.
- Arbia, W., Adour, L., Amrane, A., & Lounici, H. (2013). Optimization of medium composition for enhanced chitin extraction from *Parapenaeus longirostris* by *Lactobacillus helveticus* using response surface methodology. *Food Hydrocolloids*, 31(2), 392-403.
- Briscoe, J., Marinovic, A., Sevilla, M., Dunn, S., & Titirici, M. (2015). Biomass-derived carbon quantum dot sensitizers for solid - state nanostructured solar cells. *Angewandte Chemie International Edition*, 54(15), 4463-4468.
- Choorit, W., Patthanamance, W., & Manurakchinakorn, S. (2008). Use of response surface method for the determination of demineralization efficiency in fermented shrimp shells. *Bioresource Technology*, 99(14): 6168-6173.
- Duan, S., Li, L., Zhuang, Z., Wu, W., Hong, S., & Zhou, J. (2012). Improved production of chitin from shrimp waste by fermentation with epiphytic lactic acid bacteria. *Carbohydrate Polymers*, 89(4), 1283-1288.
- Food and Agriculture Organization of the United Nations. (2015). *Fisheries and Aquaculture*. Retrieved from <http://www.fao.org/fishery/en>
- Ghorbel-Bellaaj, O., Hmidet, N., Jellouli, K., Younes, I., Maâlej, H., Hachicha, R., & Nasri, M. (2011). Shrimp waste fermentation with *Pseudomonas aeruginosa* A2: Optimization of chitin extraction conditions through Plackett-Burman and response surface methodology approaches. *International Journal of Biological Macromolecules*, 48(4), 596-602.
- Ghorbel-Bellaaj, O., Younes, I., Maâlej, H., Hajji, S., & Nasri, M. (2012). Chitin extraction from shrimp shell waste using *Bacillus* bacteria. *International Journal of Biological Macromolecules*, 51(5), 1196-1201.
- Ghorbel-Bellaaj, O., Hajji, S., Younes, I., Chaabouni, M., Nasri, M., & Jellouli, K. (2013). Optimization of chitin extraction from shrimp waste with *Bacillus pumilus* A1 using response surface methodology. *International Journal of Biological Macromolecules*, 61(1), 243-250.
- Harish, K. V. P., & Tharanathan, R. N. (2007). Chitin/chitosan: modifications and their unlimited application potential – an overview. *Trends Food Science Technology*, 18(3), 117-131.
- Jayakumar, R., Chennazhi K. P., Muzzarelli, R. A. A., Tamura H., Nair, S. V., & Selvamarugan, N. (2010). Chitosan conjugated DNA nanoparticles in gene therapy. *Carbohydrate Polymers*, 79(1), 1-8.
- Manni, L., Ghorbel-Bellaaj, O., Jellouli, K., Younes, I., & Nasri, M. (2010). Extraction and characterization of chitin, chitosan and protein hydrolysates prepared from shrimp waste by treatment with crude protease from *Bacillus cereus*. *Applied Biochemistry and Biotechnology*, 162(2), 345-357.
- Marques, H. L. A., & Moraes-Valenti, P. M. C. (2012). Current status and prospects of farming the giant river prawn (*Macrobrachium rosenbergii* (De Man 1879) and the Amazon river prawn *Macrobrachium amazonicum* (Heller 1862)) in Brazil. *Aquaculture Research*, 43(7), 984-992.
- Ogawa, M., Maia, E. L., Fernandes, A. C., Nunes, M. L., Oliveira, M. E. B., & Freitas, S. T. (2007). Waste from the processing of farmed shrimp: a source of carotenoid pigments. *Food Science Technology*, 27(2), 333-337.
- Rao, M. S., Munoz, J., & Stevens, W. F. (2000). Critical factors in chitin production by fermentation of shrimp biowaste. *Applied Microbiology Biotechnology*, 54(6), 808-813.
- Rao, M. S., & Stevens, W. F. (2005). Chitin production by *Lactobacillus* fermentation of shrimp biowaste in a drum reactor and its chemical conversion to chitosan. *Journal of Chemical Technology Biotechnology*, 80(1), 1080-1087.
- Rao, M. S., & Stevens, W. F. (2006). Fermentation of shrimp biowaste for chitin production. *Food and Bioprocess Technology*, 44(1), 83-87.
- Rezaei, R.; Mohadesi, M., & Moradi, G. R. (2013). Optimization of biodiesel production using waste mussel shell catalyst. *Fuel*, 109(1), 534-541.
- Samelis, J., Maurogenakis, F., & Metaxopoulos, J. (1994). Characterization of lactic acid bacteria isolated from naturally fermented Greek dry salami. *International Journal of Food Microbiology*, 23(2), 179-196.
- Santos, Z. M., Caroni, A. L. P. F., Pereira, M. R., Silva, D. R., Fonseca, J. L. C. (2009). Determination of deacetylation degree of chitosan: a comparison between conductometric titration and CHN elemental analysis. *Carbohydrate Research*, 344(18), 2591-2595.
- Shirai, K., Guerreiro, I., Huerta, S., Saucedo, G., Castillo, A., Gonzalez, R. O., & Hall, G. M. (2000). Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation. *Enzyme and Microbial Technology*, 28(1), 446-452.
- Silva D., & Debacher, N. A. (2010). Caracterização físico-química e microestrutural de conchas de moluscos bivalves provenientes de cultivos da região litorânea da ilha de Santa Catarina. *Química Nova*, 33(5), 1053-1058.
- Tolaimate, A., Desbrieresb, J., Rhazia, M., & Alaguic, A. (2003). Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. *Polymer*, 44(26), 7939-7952.
- Valenti W. C. (2007) Current status of freshwater prawn culture in Brazil. In C. M. Nair, D. D. Nambudiri, S. Jose, T. M. Sankaran, K. V. Jayachandran, & K. R. Salin (Eds.), *Freshwater prawns: advances in biology, aquaculture & marketing* (105-110). New Delhi, IN: Allied Publishers.
- Vishu, A. B. K., Varadaraj, M. C., Lalitha, R. G., & Tharanathan, R. N. (2004). Low molecular weight chitosans: preparation with the aid of papain and characterization. *Biochimica and Biophysica Acta*, 1670(2), 137-146.



- Yue, W., Yao, P., & Wei, Y. (2009). Influence of ultraviolet-irradiated oxygen on depolymerization of chitosan. *Polymer Degradation and Stability*, 94(5), 851-858.
- Zhang, H., Jin, Y., Deng, Y., Wang, D., & Zhao, Y. (2012). Production of chitin from shrimp shell powders using *Serratia marcescens* B742 and *Lactobacillus plantarum* ATCC 8014 successive two-step fermentation. *Carbohydrate Research*, 362(1), 13-20.
- Zhang, H., Yang, S., Fang, J., Deng, Y., Wang, D., & Zhao, Y. (2014). Optimization of the fermentation conditions of *Rhizopus japonicus* M193 for the

production of chitin deacetylase and chitosan. *Carbohydrate Polymers*, 101(1), 57-67.

*Received on October 04, 2015.*

*Accepted on July 02, 2016.*

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.